THREE SPECIES OF BOTRYODIPLODIA (SACC.) ON ELM TREES IN THE UNITED STATES

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Plates 38 and 39 and text figure

IN CONNECTION with my work on the *Graphium* disease of elm trees in Europe, I studied some die-back diseases of Elms in the United States. Part of these investigations are related to three species of the genus *Botryodiplodia*. Two of these species were described as species of the genus *Sphaeropsis*, but were transferred by Petrak and Sydow to the genus *Botryodiplodia*. Since they are known in literature as *Sphaeropsis* species, however, I shall frequently use that name in the course of this paper, though I agree with Petrak and Sydow that their proper place is in the genus *Botryodiplodia*.

In 1920 Hubert and Humphrey described a serious die-back of American White Elm in Wisconsin. As a result of cankers extending from the twigs into the branches or into the stem, a large part of the tree or even the whole tree died. As Hubert and Humphrey always found *Sphaeropsis ulmicola* Ell. et Ev. on the cankers and always isolated this fungus from the wood of the diseased areas, they consider it the cause of the disease. Their publication, however, is only a preliminary account of the trouble, and inoculation experiments have not been made by them. They suppose that *Sphaeropsis ulmicola* Ell. et Ev. might be identical with *Sphaeropsis malorum* Peck, the well-known cause of blackrot of apples and of an apple tree canker. They pointed out the morphological similarity between the Elm Sphaeropsis and the Apple Sphaeropsis. Apples were attacked readily when inoculated with the Elm Sphaeropsis. Since the disease seems to have decreased, however, in the years following 1920, no further investigations have been carried out on the identity and the pathogenicity of *Sphaeropsis ulmicola* from a phytopathological point of view.

After I came to New England in the fall of 1929, I collected various diseased elm twigs on which a *Sphaeropsis* species was present. I decided, therefore, to continue the work of Hubert and Humphrey. During these investigations I came to the conclusion that three different species of *Sphaeropsis* may be found on elm twigs.

I. Hesler mentions the occurrence of *Sphaeropsis malorum* Peck (*Botryodiplodia malorum* [Peck] Petrak et Sydow) on twigs of *Ulmus americana*. As stated by Petrak and Sydow, the synonymy of this fungus is very complicated and not yet quite clear. It has

been thoroughly described, however, by Hesler, including its cultural characteristics, and there is little trouble in identifying it. I found pycnidia of this fungus on diseased elm specimens from various places in New England, both on two year old twigs and on older ones. As an additional aid to diagnosis of the species, I inoculated apple fruits with cultural material from certain of these specimens; typical mummies as caused by *B. malorum* from apple resulted. The bark of the diseased area in young elm twigs is depressed, and separated by a rather sharp edge from the healthy tissue, thus forming a canker. The whole twig may be attacked, or the cankered area may extend along one side of the twig only. Older branches are sometimes flattened as a result of the attack. Below the diseased area twigs may grow somewhat after the manner of a witches'-broom. The diseased wood assumes a brown color, and brown streaks may extend for some distance into the healthy wood. These symptoms may be found both on nursery stock and on large trees. The twigs above a cankered area often lose their leaves and die. From the diseased wood *Sphaeropsis malorum* can be isolated. Sometimes pycnidia of *Phomopsis* sp. and *Cytospora* sp. are also found on these cankers.

When *Sphaeropsis malorum* is grown on sterilized elm twigs it often forms pycnidia with spores. It loses its spore-forming capacity, however, after it has been transferred a few times. I made various one-spore cultures of this fungus. As the spores are fairly large, it is easy to fish them with a thin glass needle from a drop of water and to deposit them on a clear agar medium in a petri dish. Under such circumstances germination of the spores can be quite readily observed. Hesler stated that the sizes of the spores and the relative numbers of two-celled spores are different in various isolations, and I found the same to be true. I measured 50 spores each from elm and apple twigs and from cultures (see figs. 1–5), and found the following sizes:

1. Elm twig: 21–24 μ x 10–12 μ;
2. Apple twig: 20–23 μ x 10½–13 μ;
3. Culture from elm twig: 23–26 μ x 10–12 μ;
4. Culture from apple twig: 22–25 μ x 9–11 μ.

Some inoculation experiments were carried out with *Sphaeropsis malorum*, isolated from elm twigs, on young trees about thirty inches high of *Ulmus americana* growing in the greenhouse. Cross inoculations were also made with the same fungus that had been isolated from an Elm and cultured on an Apple. The inoculations were performed by transferring some mycelium from a pure culture of *S. malorum* into a T-cut in the stems of elm saplings and into

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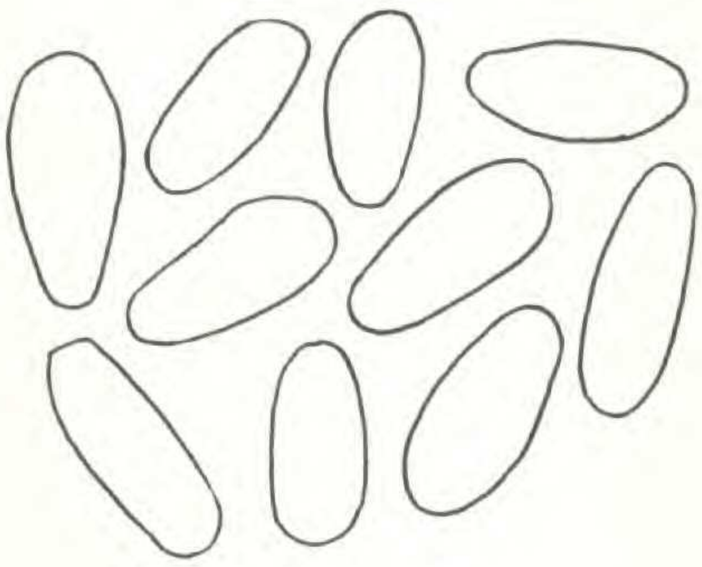

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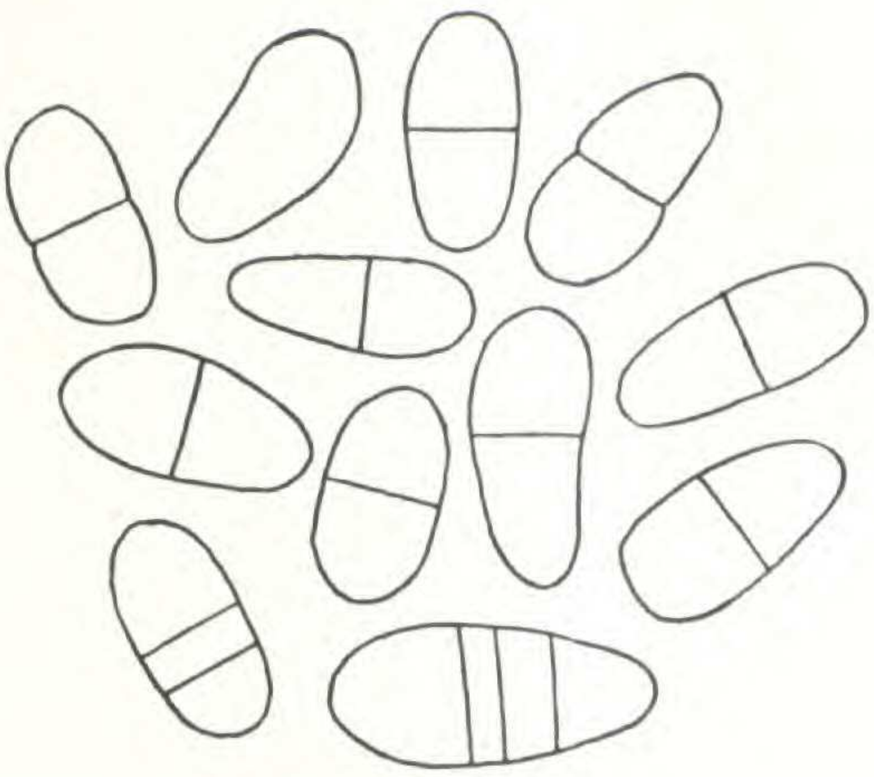

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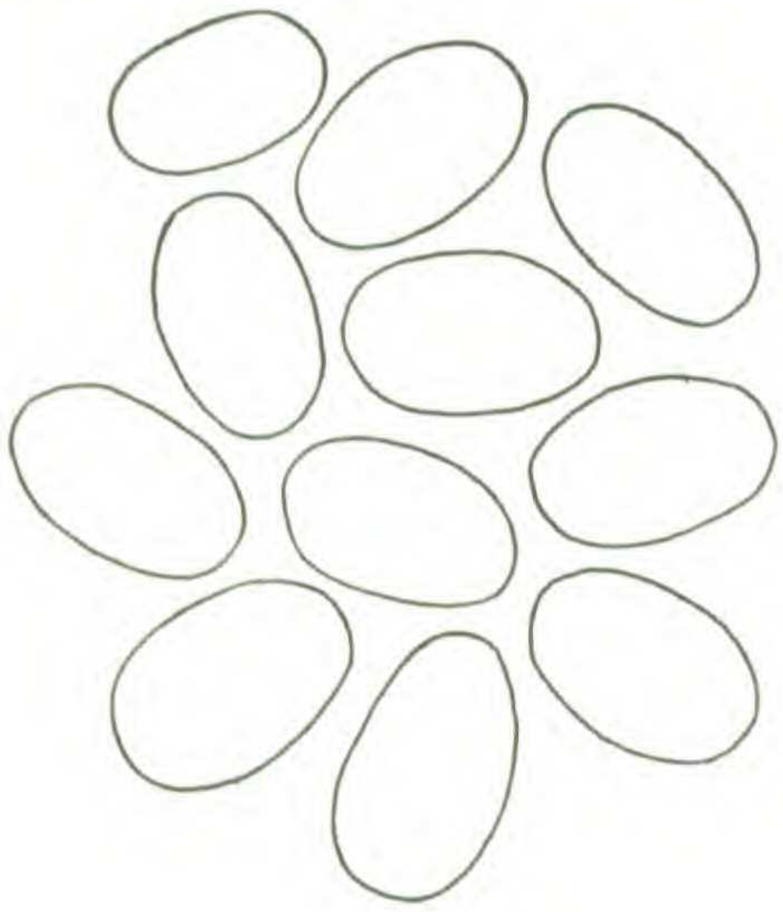

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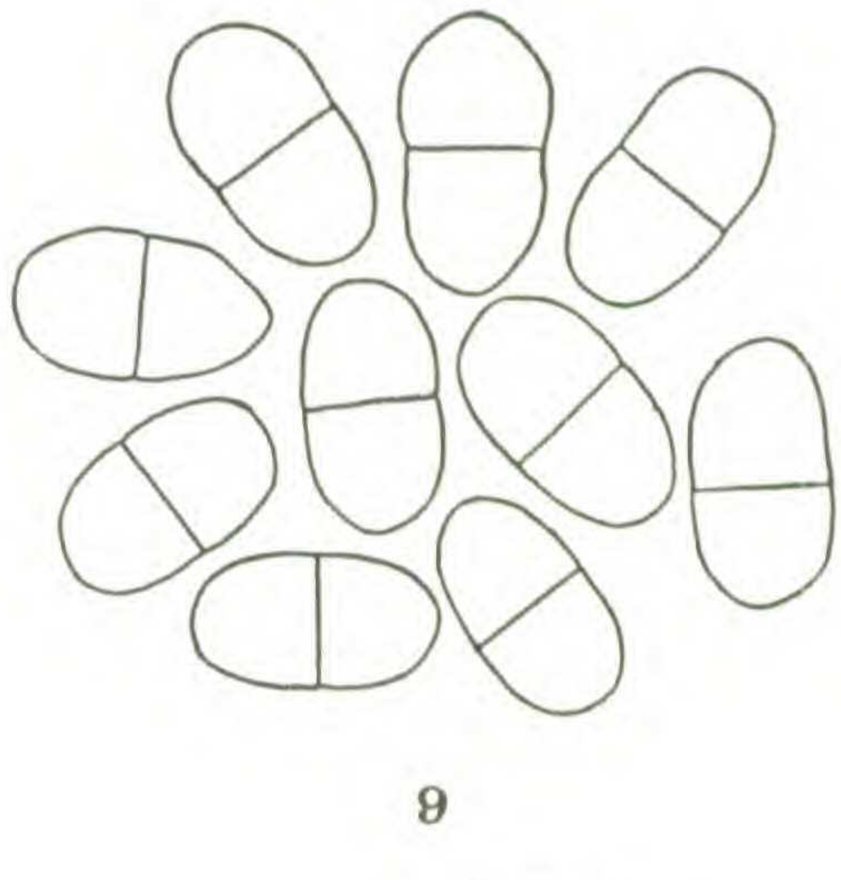

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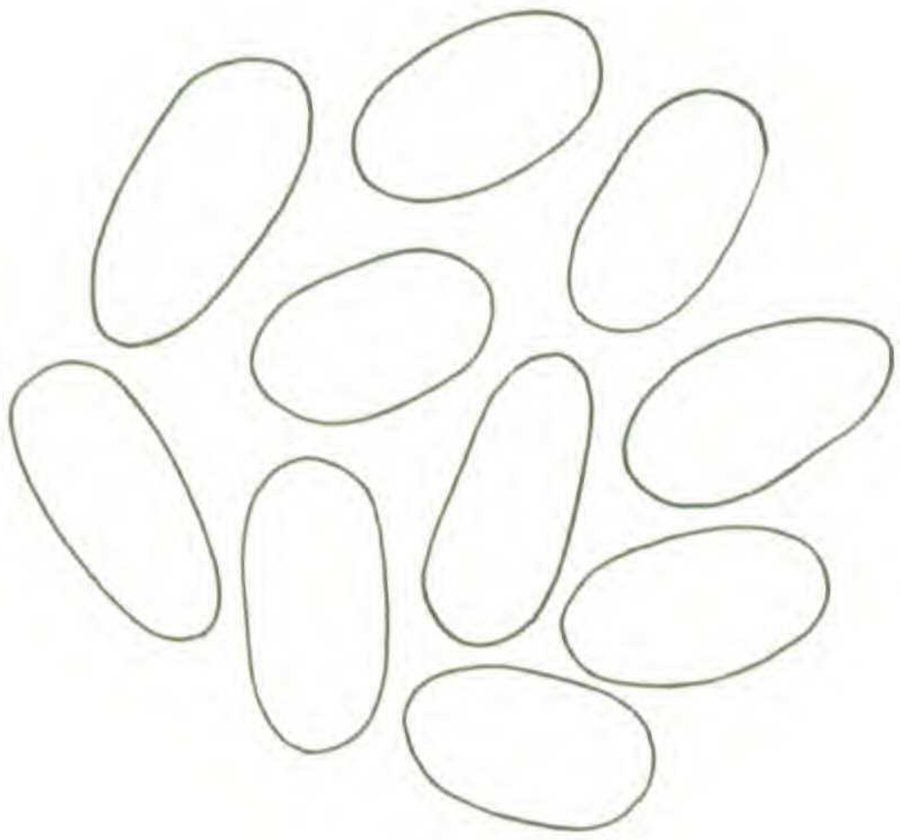

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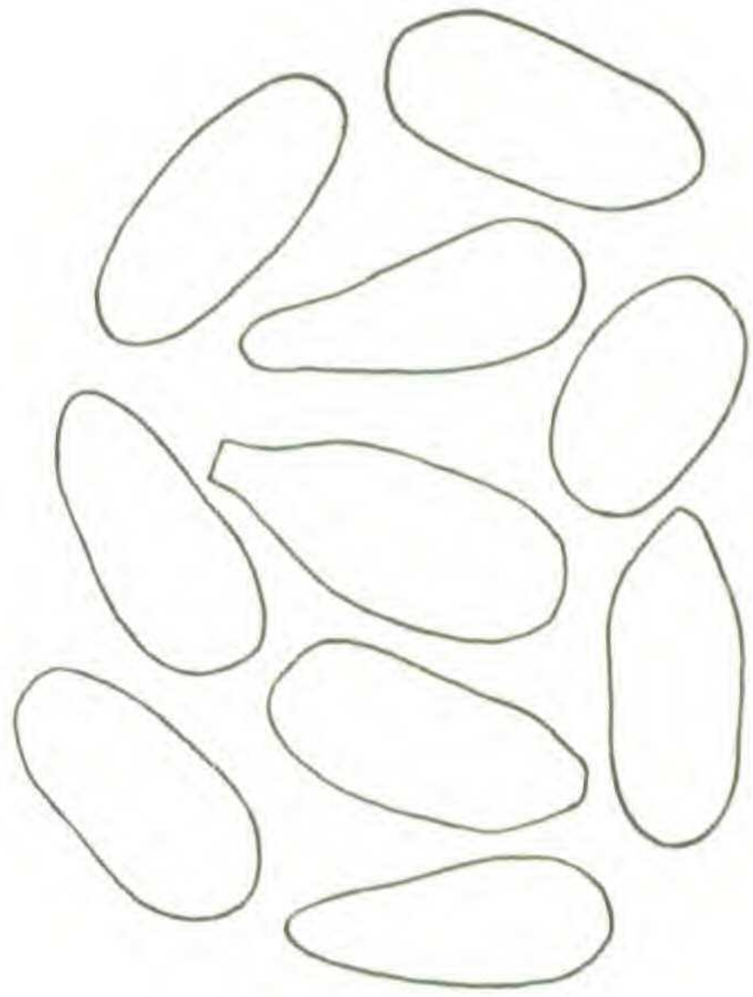

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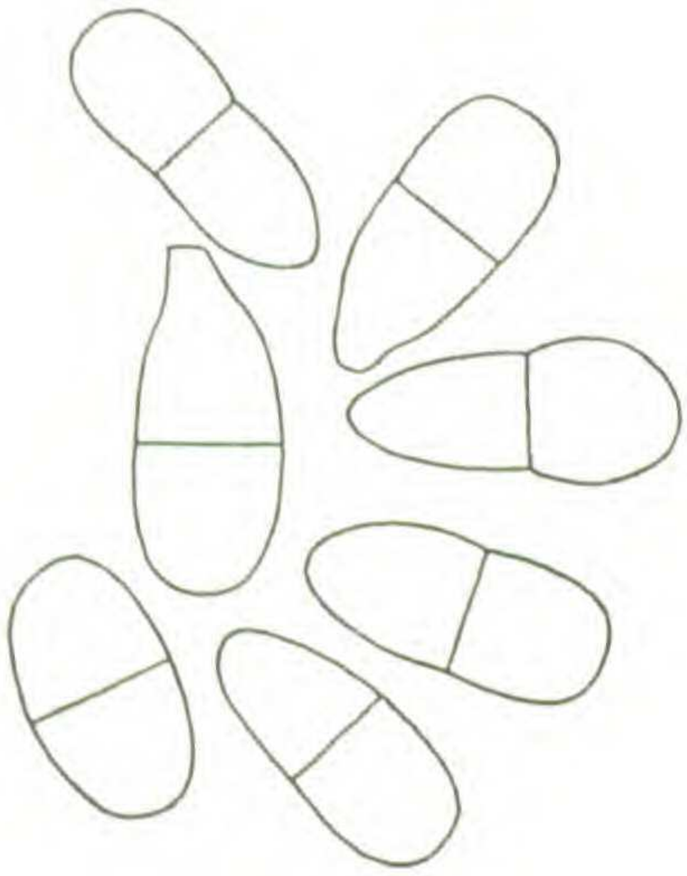

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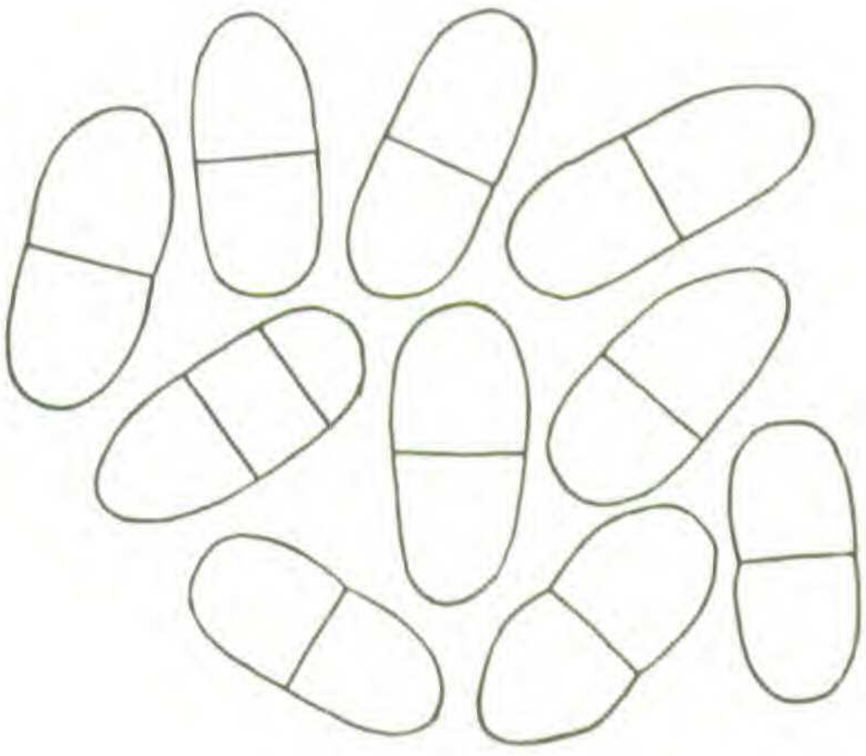

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apple twigs. After each inoculation the wound was protected by raffia.

Though Hesler states that a canker formation resulted on apple bark after inoculation with *Sphaeropsis malorum*, originating from *U. americana*, my experiments were negative. On August 20, 1930, young trees, inoculated November and December 1929, and February 1930, were examined. There was neither a formation of cankers nor brown discoloration of the wood. Small pieces of some of these young trees were excised just where the inoculations had been made; the pieces removed were sterilized by leaving them in 0.1% corrosive sublimate for some minutes. After this the bark was peeled off and the pieces of wood were placed in a petri dish with agar. A fungus, always of the same type, grew out from these pieces of wood in six of the eight cases tested; this fungus must be assumed to be *S. malorum*, for while it did not at once form any pycnidia, its vegetative growth was similar to that of *S. malorum*. Three of these cultures that were transferred and taken to Holland have since produced typical spores of *S. malorum* when grown on sterilized maple twigs.

My inoculation experiments, therefore, do not prove the pathogenicity of *Sphaeropsis malorum* with regard to Elms. Since, however, the experiments were performed in a greenhouse, it may be that the conditions were not suitable for infection. In any case, *S. malorum* does not seem to be a virulent parasite for Elms. It is possible that it can establish itself in the twigs only after they have been weakened by some other cause.

II. I stated above that a canker on American White Elm, ascribed to *Sphaeropsis ulmicola* Ell. et Ev. on American Elm, has been reported by Hubert and Humphrey from Wisconsin. Some twigs, showing the typical symptoms of this disease, were sent to me from Wisconsin by Dr. Audrey Richards. Though no pycnidia were present on those twigs it was very easy to isolate *Sphaeropsis ulmicola* from them. Spores were produced in subsequent cultures on sterilized elm twigs. I found that in the same culture dark or white spore horns might be seen protruding from the pycnidia. The white spore horns consist of hyaline, continuous spores, the dark ones of dark brown, mostly two-celled spores. When one-spore cultures were made using either white or brown spores, the results were exactly the same. In the cultures, grown either from a white or from a brown spore, white and brown spore horns might develop regardless of the original color of the spores planted. It may be stated incidentally that agar media are not so suitable for the production of spores as sterilized elm twigs. The growth on elm twigs is dark, and only comparatively few pycnidia are formed.

I measured fifty white and fifty brown spores (see Figs. 6 and 7), and the results were

White spores: 27–30 μ x 14–15 μ;

Brown spores: 25–30 μ x 12–15 μ.

The spores are, therefore, decidedly larger and especially broader than those of *Sphaeropsis malorum*.

Petrak and Sydow include *Sphaeropsis ulmicola* under *Botryodiplodia hypodermia* (Sacc.) Petr. and Syd. In my opinion this should not be done. The fungus I isolated from the Wisconsin twigs agrees closely with the description Ellis and Everhart give of *Sphaeropsis ulmicola*. As the description of Petrak and Sydow of *Botryodiplodia hypodermia* agrees with the third species of *Sphaeropsis* that I found on elm twigs, I propose the removal of *Sphaeropsis ulmicola* Ell. et Ev. from *Botryodiplodia hypodermia* (Sacc.) Petr. et Syd., and prefer to call it **Botryodiplodia ulmicola** (Ell. et Ev.), nov. comb.

Some inoculation experiments were carried out with *S. ulmicola* on young elm trees, in the same way as with *S. malorum*. The inoculations were made in November and December 1929, and in January, February and April 1930, always in the greenhouse. On August 20, 1930, several inoculated saplings were examined, but only in two cases out of twenty-six was a slight discoloration of the wood visible. Six small pieces of these saplings, excised just at the region of inoculation, were sterilized, peeled and placed in a petri dish with agar, as has been described already. In all cases a fungus, similar in its vegetative growth to *S. ulmicola*, was isolated from the wood. Though no spores were immediately produced two of the transfers of these cultures that were taken back to Holland have since produced typical spores of S. *ulmicola*. In two instances small pieces of the saplings, taken just above the points of inoculation, were treated in the same way, but no fungus similar to *S. ulmicola* could be isolated. Therefore, while the fungus was apparently still alive in the wood, it did not make any progress in the suscept beyond the inoculation court.

III. A third species of *Botryodiplodia* I found only on dead twigs of *U. foliacea suberosa* in the Arnold Arboretum of Harvard University. A great many of the young twigs of this tree had died, but this apparently did not seriously interfere with the vigor of the tree, as immediately below the dead twigs new ones were formed, and the trouble did not spread to the thicker twigs. On the dead twigs pycnidia were present, filled with spores. It was easy to fish these spores from a drop of water with a glass needle and deposit them in a petri dish. They germinated readily and soon the